Denitrification in the Upper Mississippi River: rates, controls, and contribution to nitrate flux

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Abstract: We evaluated patterns of denitrification and factors effecting denitrification in the upper Mississippi River. Measurements were taken over 2 years, during which river discharge ranged from record flooding to base flow conditions. Over the period of study, average denitrification enzyme activity was highest in backwater lakes and lowest in the main channel. Throughout the study reach, highest denitrification enzyme activity occurred during fall and lowest occurred in winter. Rates during spring floods (2001) were only slightly higher than during the preceding winter. Mean unamended denitrification rates ranged from 0.02 (fall 2001 in backwaters) to 0.40 μg $N \cdot cm^{-2} \cdot h^{-1}$ (spring 2001 in backwaters). Laboratory experiments showed that denitrification rates increased significantly with addition of NO_3^- regardless of sediment C content, while rates increased little with addition of labile C (glucose). Denitrification in this reach of the upper Mississippi River appears to be NO_3^- limited throughout the growing season and the delivery of NO_3^- is strongly controlled by river discharge and hydrologic connectivity across the floodplain. We estimate that denitrification removes 6939 t $N \cdot year^{-1}$ or 6.9% of the total annual NO_3^- input to the reach. Hydrologic connectivity and resultant NO_3^- delivery to high-C sediments is a critical determinant of reach-scale processing of N in this floodplain system.

Résumé : Nous avons évalué les patterns de dénitrification et les facteurs qui opèrent la dénitrification dans le Mississippi supérieur. Les mesures ont été réalisées sur 2 années, pendant lesquelles le débit de la rivière a varié d'inondations record à des conditions d'étiage. Durant la période d'étude, l'activité enzymatique moyenne de dénitrification était maximale dans les lacs de la plaine de débordement et minimale dans le chenal principal. Dans toute la zone d'étude, les valeurs maximales de l'activité enzymatique moyenne de dénitrification ont été mesurées à l'automne et les valeurs minimales en hiver. Les taux durant les inondations du printemps (2001) étaient tout juste un peu plus élevés que l'hiver précédent. Les taux moyens non corrigés de dénitrification variaient de 0,02 (automne 2001 dans des eaux de la plaine de débordement) à 0,40 μg $N \cdot cm^{-2} \cdot h^{-1}$ (printemps dans des eaux de la plaine de débordement). Des expériences en laboratoire montrent que les taux de dénitrification augmentent de façon significative après l'addition de NO_3^-, quel que soit le contenu des sédiments en C; ces taux augmentent peu après l'addition de C labile (glucose). La dénitrification dans cette section du Mississippi semble être limitée par NO_3^- durant la saison de croissance et l'apport de NO_3^- est fortement contrôlé par le débit de la rivière et la connectivité hydrologique à travers la plaine de débordement. Nous estimons que la dénitrification retire 6 939 t $N \cdot an^{-1}$, soit 6,95 % de l'apport annuel de NO_3^- dans la section. La connectivité hydrologique et l'apport de NO_3^- aux sédiments riches en C qui en résulte sont des facteurs déterminants essentiels du traitement de l'azote à l'échelle de la section dans ce système de plaine de débordement.

[Traduit par la Rédaction]

Introduction

Floodplain rivers carry a substantial fraction of the dissolved nutrients lost from large catchments (Caraco and Cole 1999). The Mississippi River drains nearly 40% of the continental United States, delivering an average of nearly 1×10^6 tonnes (t) $NO_3^- \cdot year^{-1}$ to the Gulf of Mexico (Goolsby and Battaglin 2001). Estimates of N loading and transport by the Mississippi River suggest that N moves conservatively from upland sources to the Gulf. Such unimpeded nutrient loading to marine environments has contributed to eutrophication and hypoxic conditions in nearshore zones of the Gulf of Mexico (Rabalais and Turner 2001). Modeling by Alexander et al. (2000) indicates that greater than 90% of

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the NO_3^- reaching the Mississippi River will be transported to the Gulf of Mexico. This implies that the Mississippi River is merely a nonreactive transport conduit, with little processing or removal of N in transit. Further, analysis of water chemistry and stable isotopes of N (^{15}N) and oxygen (^{18}O) of NO_3^- in water from the middle and lower Mississippi River suggests that little, if any, N is lost in transit and transformations of N are due mainly to assimilation and not denitrification (Battaglin et al. 2001). Paradoxically, the backwater lakes and riparian wetlands associated with the upper Mississippi River (UMR) contain optimal conditions for removal of NO_3^- through microbial denitrification because of highly organic, anoxic sediments and abundant rooted macrophytes. Under conditions of high NO_3^- loading to organic sediments, denitrification should effectively remove a detectable portion of NO_3^- loads. Statistical models suggest that NO_3^- loss in the UMR is low, but a lack of direct estimates of N cycling rates or processes hinders explanation.

Nitrogen transformations do occur in large temperate zone rivers at significant rates. For example, in the Wiske–Swale–Ouse River system (United Kingdom), rates of denitrification tend to increase from headwater to estuary, with increasing NO_3^- concentration and temperature (Garcia-Ruiz et al. 1998*a*; Pattinson et al. 1998). Direct estimates of high rates of N_2O release, combined with mass balance modeling, suggests that denitrification occurs at elevated rates, particularly during warm summer months, in the South Platte River, Colorado (Sjodin et al. 1997). In general, large rivers can remove anywhere from 5% to 20% of their NO_3^- loads (Seitzinger 1988) through denitrification.

In NO_3^--limited environments, denitrification may be dependent on, and tightly coupled with, nitrification (microbial oxidation of NH_4^+ to NO_3^-; Caffrey and Kemp 1992). Process coupling may be important in backwater areas of the UMR where water column NO_3^- concentrations may be undetectable (D.M. Soballe, unpublished data). Thus, a complete understanding of the spatial and temporal patterns of denitrification in the river sediments also requires knowledge of the patterns of sediment nitrification.

Here, we present the first systematic evaluation of spatial and temporal patterns of denitrification in a large reach (Navigation Pool 8) of the UMR near La Crosse, Wis. The reach is typical of many in the UMR, containing flowing channels, impounded zones, and relatively isolated backwater lakes and riparian wetlands. Our work is an initial attempt to understand factors regulating N cycling in a large temperate floodplain river where connection with a historic floodplain complex still exists. This river ecosystem is highly modified for navigation and lateral flood control, but unlike the lower half of the Mississippi River, there is still active connection between flowing channels, off-channel floodplain lakes and marshes, and impoundments.

Our objectives were to determine (*i*) spatial variability of sediment denitrification and associated environmental conditions (e.g., water column NO_3^- concentrations, sediment C and N content, and river discharge), (*ii*) temporal variation in these spatial patterns, (*iii*) coupling between nitrification and denitrification, (*iv*) substrate (C and NO_3^-) limitation of denitrification in river sediments, and (*v*) contribution of denitrification to the reach NO_3^- budget.

Materials and methods

Site description (Navigation Pool 8)

The Mississippi River above St. Louis, Mo., is divided by a series of low head dams into 27 reaches to facilitate navigation. Pools range from 9.3 to 74.5 km long. Navigation Pool 8 (43.75°N, 91.25°W) near La Crosse, Wis., is 37.5 km long with a median discharge of 815 $m^3 \cdot s^{-1}$ and contains 10 425 ha of wetted area under normal summer flows (Fig. 1). Average depth across pool is 1.7 m, with a depth of at least 3.8 m in the main channel. During nonflood periods, the bulk of the water flowing through the pool remains in the main channel aided by flow-directing structures (wing dams and side channel closing dams) and channel dredging.

Pool-wide sampling

We sampled across four main categories of aquatic areas: impounded, contiguous backwater, side channel, and main channel (Table 1). The aquatic area categories are standardized across all pools of the UMR and provide a basis of comparison within and among other pools of the entire UMR (Wilcox 1993). Sediment and surface water were sampled at 60 or more sites during six periods: May (spring), August (summer), and October (fall) 2000 and May, July–August, and October 2001. In January (winter) 2001, we only sampled 15 sites because of difficulties of winter sampling. These sample periods correspond to "seasons" that are considered 3-month time spans and encompass critical historical patterns in river discharge and water temperature. Surface water was collected at each site for analysis of NH_4^+ and NO_3^- + NO_2^- (hereafter referred to as NO_3^-) after filtration (Whatman 0.45-μm-mesh glass fiber filter). Total N was determined in unfiltered samples. Samples were acidified (pH < 2) with H_2SO_4 and stored at 4 °C for later analysis. NH_4^+ concentration was determined using the automated phenate method; total N (with persulfate digestion) and NO_3^- were determined with the automated Cd reduction method on a Bran+Luebbe continuous flow analysis system using standard methods (American Public Health Association 1998). Minimum detection limits for nutrient analyses were 0.016 mg $N \cdot L^{-1}$ for surface water NO_3^- and total N, 0.01 mg $N \cdot L^{-1}$ for NH_4^+, and 0.01 mg $N \cdot L^{-1}$ for porewater NH_4^+. Water temperature, dissolved oxygen, pH, and conductivity were measured in situ with a YSI 600XL multiprobe. Average river discharge, water temperature, dissolved oxygen, NO_3^-, and NH_4^+ concentrations for each sample period are given in Table 2. Sediment cores (7.62 cm in diameter × 5 cm) were taken at each site; pH and temperature of sediments were measured immediately after collection with a Beckman Φ pH meter and then refrigerated for later processing. Sediment total C (mass loss on ignition), volatile mass, bulk density, and percent water mass were determined from homogenized subsamples following standard methods (American Public Health Association 1998). Sediment porewater was removed with centrifugation at 3000 rpm for 12 min and analyzed for NH_4^+ and NO_3^- as described previously. Total exchangeable sediment NH_4^+ was determined following Caffrey and Kemp (1992).

Denitrification measurements

Three metrics were used to quantify microbial denitri-

Fig. 1. Location of the study reach (Navigation Pool 8) near La Crosse, Wis.

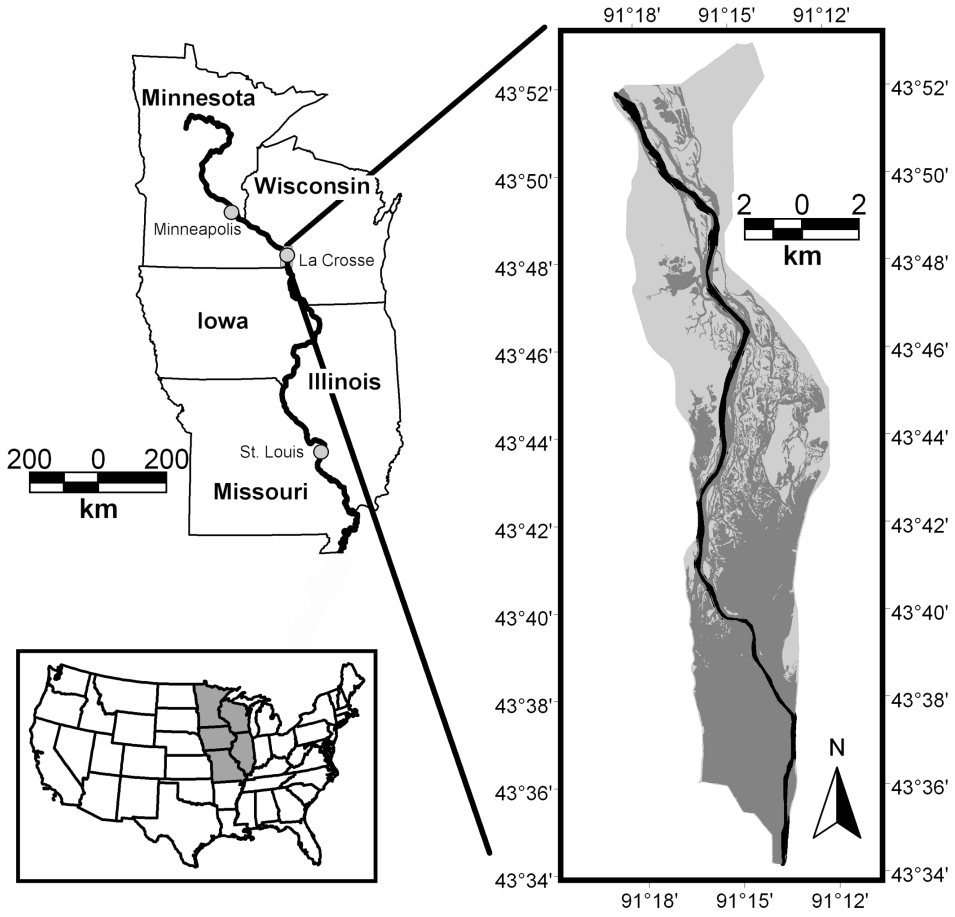


Table 1. Surface area and sediment characteristics of aquatic areas in Navigation Pool 8.

Aquatic area type	Total area in pool (km^2)	Fraction of total pool area (%)	Sediment bulk density (SE) ($g \cdot cm^{-3}$)	Volatile C (SE) ($g \cdot g$ dry $sediment^{-1}$)
Impounded	36.9	45	1.63 (0.018)	0.16 (0.016)
Backwater	19.4	24	1.24 (0.017)	0.16 (0.011)
Side channel	13.2	16	1.87 (0.03)	0.09 (0.036)
Main channel	12.6	15	1.88 (0.055)	0.05 (0.005)
Total	82.1	100		

fication in river sediments: (*i*) denitrification enzyme activity (DEA) in 2000 and 2001, (*ii*) unamended denitrification (U-DEN) in 2001, and (*iii*) estimated denitrification rate (EDR) in 2001. DEA and U-DEN were determined using variations of the acetylene block technique (Sorensen 1978; Tiedje et al. 1989) and EDR was a calculated metric using DEA, U-DEN, and nitrification rate (Strauss et al. 2004) values. All rate estimates are reported on an aerial basis, consistent with data reported by many similar studies (see Seitzinger 1988), but because original sediment cores and slurried subsamples were of standardized volume, shape, and depth, rates analyzed on an aerial or volume basis resulted in no difference in final interpretation.

DEA was determined in sediments from all sites following Groffman et al. (1999) and is a standard assay to determine activity rates of extant denitrifying enzymes, given unlimited organic C and NO_3^- substrate. The DEA technique employed here is a useful tool for relative comparisons of denitrification across aquatic areas and seasons because it minimizes the high variation in rate estimates commonly associated with short-term substrate limitation (Groffman et al. 1999). However, DEA rates are often higher than actual rates of denitrification because of substrate amendments; therefore, DEA rates should not be considered as actual ambient rates. Within 24 h of extraction from the river bottom, 25 mL of sediment from the upper 5 cm of cores (2.54 cm in diameter) was slurried with additions of 20 mL of sample site water and 5 mL of DEA solution (final concentrations: 12 mg glucose-$C \cdot L^{-1}$, 14 mg NO_3^--$N \cdot L^{-1}$, and 100 mg $chloramphenicol \cdot L^{-1}$). This chloramphenicol concentration is adequate to inhibit production of new nitrate reductase without inhibiting the function of existing enzymes (Murray and Knowles 1999). Seasonal variation in the magnitude of coupling between nitrification and denitrification was assessed

Table 2. Sample events and dates, mean discharge (Q) at Lock and Dam 8 and surface water temperature, surface and bottom dissolved oxygen, surface NO_3^-, and surface NH_4^+ in Navigation Pool 8.

			Surface temperature (°C)		Surface dissolved oxygen (mg O_2·L^{-1})		Bottom dissolved oxygen (mg O_2·L^{-1})		NO_3^- (mg N·L^{-1})		NH_4^+ (mg N·L^{-1})	
Sample event	Sample dates	Q (m^3·s^{-1})	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Spring 2000	2–16 May	754	18.0	15.4	11.1	26.0	10.1	34.0	0.18	197.5	0.05	74.5
Summer 2000	31 July – 8 August	737	23.2	8.1	6.2	40.2	5.7	46.0	1.64	80.3	0.13	78.5
Fall 2000	31 October – 9 November	687	11.4	23.6	10.2	14.5	10.2	18.8	0.57	103.1	0.09	73.6
Winter 2001	14–23 February	556	0.2	115.3	11.2	37.7	9.7	56.3	1.46	53.1	0.28	98.5
Spring 2001	15–21 May	3063	18.9	9.4	8.9	10.1	8.6	9.5	2.61	25.0	0.06	68.6
Summer 2001	24 July – 2 August	844	27.2	5.1	9.6	39.0	8.8	28.8	0.71	60.5	0.12	157.7
Fall 2001	1–10 October	448	14.9	14.5	9.4	23.2	9.5	23.8	0.73	71.2	0.05	59.0

using linear regression of DEA with nitrification (Strauss et al. 2004).

After slurries were prepared, anaerobic conditions were initiated through purging of sample jars of oxygen with scrubbed, ultra-high-purity helium for 15 min. Atomic absorption grade acetylene (20 mL) was then added with a syringe through a septum on the top of each sample container. Slurries were incubated, under constant agitation (175 rpm), at ambient river temperatures in a darkened incubator. Headspace gas was sampled at 30, 60, and 90 min and N_2O concentrations were measured using a Hewlett–Packard model 5890 gas chromatograph with an electron capture detector (ECD ^{63}Ni).

U-DEN rates were determined during spring, summer, and fall 2001. Methods were identical to those for DEA except there were no additions of DEA solution to the sediments and N_2O samples were taken at 1, 4, and 24 h. Converse to DEA rates, U-DEN rates can underestimate actual denitrification rates because NO_3^- production (i.e., nitrification) is inhibited during the incubation period by acetylene (Hynes and Knowles 1978) and the anaerobic environment. In this study, U-DEN was considered as an extremely conservative estimate of denitrification and was useful in examining the hypothesis that variation in river discharge redistributes NO_3^--rich waters and stimulates denitrification rates in normally NO_3^--poor aquatic areas (e.g., contiguous backwaters).

Because denitrification in this system is limited by NO_3^- availability in the sediments, the actual denitrification rate ranges between the rates of DEA and U-DEN. EDR is an estimate of actual denitrification rate within the limits set by the DEA and U-DEN determinations. For spring, summer, and fall 2001, EDR was calculated as the more conservative (lower) value between DEA rate and U-DEN rate plus nitrification rate (Strauss et al. 2004). A linear relationship between EDR and rates of nitrification and DEA was determined with linear regression (EDR = 0.7393(nitrification rate) + 0.04160(DEA rate) + 0.02192; R^2 = 0.755) and used to calculate EDRs for spring, summer, and fall 2000 and winter 2001. EDR is essentially an estimate of the actual denitrification rate existing at a site in the presence of nitrification and its validity rests in three assumptions: (*i*) denitrification is limited by NO_3^- availability, (*ii*) denitrification is not limited by C, and (*iii*) heterogeneous availability of oxygen in the sediments to allow for concurrent nitrification and denitrification. Heterogeneous availability of oxygen is a common sediment phenomenon, especially within photosynthetic mats (Glud et al. 1999) and in areas with rooted macrophytes (Moorhead and Reddy 1988). This phenomenon has also been documented within the UMR system and discussed by Strauss et al. (2004). EDR was used in this study primarily for calculating the contribution of denitrification to overall NO_3^- loss in the UMR Pool 8 NO_3^- budget.

Limitation experiment

We conducted a controlled experiment to determine if C and (or) NO_3^- availability limits sediment denitrification in Pool 8 and if limitation varies by sediment total C content. Four sediment cores (top 5 cm) were taken from each of three sediment types: high-C (0.12 g C·g sediment^{-1}) backwater, intermediate-C (0.036 g C·g sediment^{-1}) areas, and low-C (0.008 g C·g sediment^{-1}) side channel. Sediment was

placed in jars and formed into slurries with water from the site. NO_3^- treatments were dosed with NO_3^- to a final concentration of 2 mg NO_3^--N·L^{-1}, C treatments were amended with glucose to a final concentration of 20 mg glucose-C·L^{-1}, the combined treatments were amended to final concentrations of both 2 mg NO_3^--N·L^{-1} and 20 mg glucose-C·L^{-1}, and controls consisted of sediments with no further additions. Denitrification was measured using the acetylene block method described above.

Pool NO_3^- mass balance

The contribution of denitrification to pool-wide NO_3^- flux was estimated by first calculating seasonal area NO_3^- losses in aquatic areas as the product of mean seasonal EDRs for each aquatic area and the total surface area of the respective aquatic area (with appropriate time conversions). Next, seasonal pool-wide NO_3^- losses were calculated as the sum of the seasonal aquatic area NO_3^- losses. Finally, annual intrasystem NO_3^- loss through denitrification was estimated as the sum of the seasonal pool-wide estimates. Measurement error also was extrapolated from initial estimates to pool-wide NO_3^- production values using appropriate error summation and conversion formulas (Pitman 1993). Using data from Strauss et al. (2004), we then determined the annual NO_3^- budget for Pool 8; inputs include UMR mainstem and tributary loads and nitrification, and outputs include mainstem downstream load, denitrification, and an unquantified "other" estimated through mass balance. Inputs from groundwater, relative to upstream loading, were negligible (R. Hunt, US Geological Survey, 8505 Research Way, Middleton, WI 53562, USA, unpublished data).

Data analysis

We tested the null hypothesis of no differences in DEA and U-DEN rates among aquatic areas, seasons, and years (when appropriate) with fixed effects general linear model analysis of variance (Littell et al. 1996). Residuals output from the models indicated that the assumption of homoscedasticity was not met and, therefore, the data were fourth-root transformed and the analysis was then conducted on the transformed data (Sokal and Rohlf 1981). We evaluated the magnitude of the temporal and spatial covariance by creating semivariogram plots from residuals of the general linear models. Semivariogram plots were useful in interpreting the lag of temporal "distance" between sampling events or lag distance (metres) between sampling sites for the temporal or spatial covariance analysis, respectively. We did not detect temporal correlation but did observe spatial correlation at distances of less than 300 m; however, this covariation was represented by the semivariance in only six or fewer pairs (of several thousand possible pairwise comparisons) of sites for a given sampling event. Aquatic areas within a given reach (navigation pool) of the UMR have been shown to be independent for most water quality variables sampled by the Long Term Resource Monitoring Program (B. Gray, US Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI 54603, USA, personal communication) but nested among reaches. To determine differences among means, we used the least significant difference test for unplanned comparisons of means, corrected for inflated comparison-wise error rates with the Bonferroni correction (Littell et al. 1991).

Environmental variables showing significant correlations with DEA and U-DEN were entered into stepwise linear regression models to determine predictive relationships. Entry and retention of variables into each model was set at a conservative level of $P = 0.05$ to reduce the likelihood of inflated comparison-wise error rates (Littell et al. 1991). Relationships among mean responses of denitrification from the limitation experiment were analyzed using general linear model analysis of variance (Littell et al. 1991). In the limitation experiment, we tested the null hypothesis that neither the treatments (NO_3^- or C supplements) nor the combination of supplements had an effect (not different from controls) on denitrification rate. We also tested the null hypothesis that site-specific sediment C content had no effect on denitrification responses to NO_3^- and C supplements. Both sets of tests were conducted with two-way analysis of variance (general linear models) (Littell et al. 1991).

Results

Spatial and temporal distribution of NO_3^-

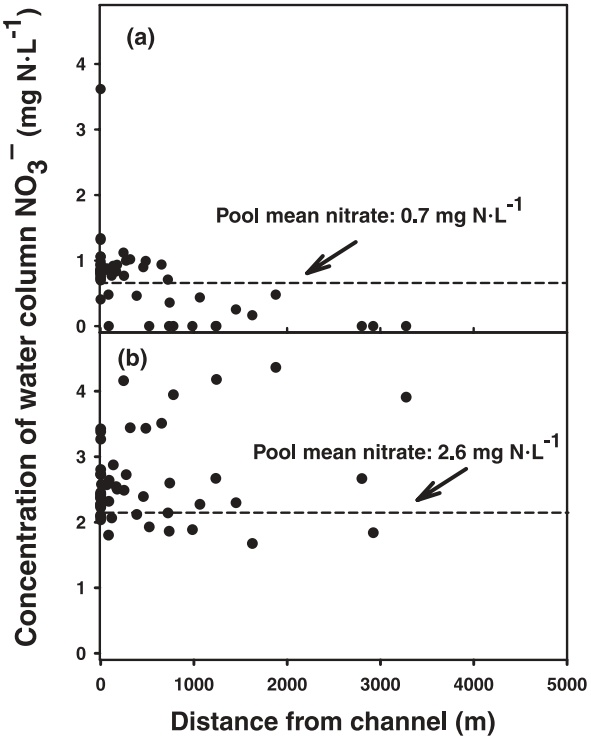
NO_3^- concentrations varied in Navigation Pool 8 depending on aquatic area, season, and river discharge. The main channel generally contained the highest concentrations of NO_3^- (mean 1.72 mg·L^{-1}, maximum 7 mg·L^{-1}), while backwaters were typically the lowest (mean 0.66 mg·L^{-1}, maximum 4.4 mg·L^{-1}). Concentrations in the impounded areas tended to be intermediate to those in the main channel and backwaters (mean 1.3 mg·L^{-1}, maximum 3.51 mg·L^{-1}). Side channel concentrations were highly variable but tended to be slightly lower than those in impounded areas (mean 1.18 mg·L^{-1}, maximum 2.77 mg·L^{-1}).

Spatial distribution of NO_3^- concentrations varied with season, a pattern strongly influenced by river discharge. NO_3^- concentrations tended to diminish with distance from a flowing channel during nonflood periods and equalize across the pool during floods (Fig. 2). For example, during the base flow conditions from fall 2001, 47% of the variance in NO_3^- concentration across Pool 8 was accounted for by the distance from a flowing channel ($P < 0.0001$), with most backwater sites exhibiting undetectable NO_3^- concentrations. In fact, nearly 10% of all sites sampled during fall 2001 had concentrations below detection limits (<0.016 mg·L^{-1}), a surprising result for a river system with relatively high average NO_3^- concentrations. During spring flooding (spring 2001), NO_3^- concentrations were unrelated to distance from the main channel. It was also during spring flooding that the highest pool-wide NO_3^- concentrations were observed (mean 2.61 mg·L^{-1}) and spatial distribution was most homogenous (CV = 25.0) (Table 2). In comparison, there was no flood the previous spring (May 2000) and NO_3^- concentrations were lower (mean 0.18 mg·L^{-1}) and extremely variable (CV = 197.5).

Sediment characteristics

Sediment characteristics reflected the erosional patterns of flowing waters (Table 1): sediments in the slack water areas (backwaters and impounded) contained significantly more volatile C ($F_{[3,374]} = 6.66$, $P = 0.002$) than flowing areas (side and main channel). Volatile C of sediments from across

Fig. 2. Concentration of water column NO_3^- by distance of sample point from a flowing channel in Navigation Pool 8 during (*a*) fall 2000 (base flow conditions) and (*b*) spring 2001 (flood conditions).



the reach were significantly higher ($F_{[1,374]}$ = 18.6, P < 0.0001) in 2001 (mean ± 1 SE = 0.109 ± 0.0004) than in 2000 (0.137 ± 0.0009). The greatest change occurred in the impounded (2000 versus 2001: 0.11 versus 0.18 g·g dry sediment^{-1}) and backwaters (2000 versus 2001: 0.14 versus 0.16 g·g dry sediment^{-1}) and in summer (2000 versus 2001: 0.13 versus 0.17 g·g dry sediment^{-1}).

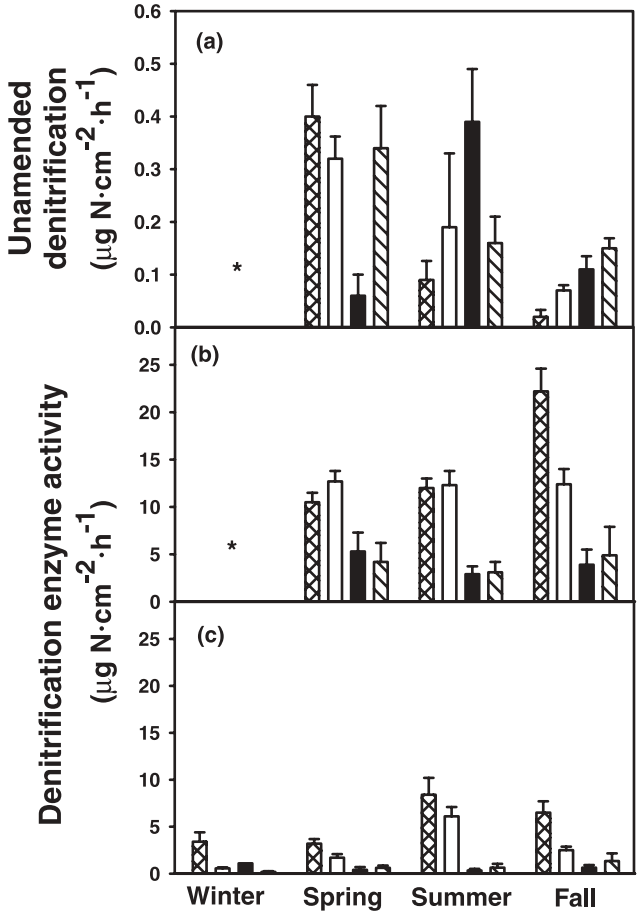
Sediment bulk density was significantly lower in backwaters, intermediate in the impounded zone, and highest in the channels ($F_{[3,374]}$ = 230, P < 0.0001). These differences result from greater sand content in sediments from flowing areas.

Denitrification estimates

DEA varied significantly among aquatic areas (ANOVA, $F_{[3,335]}$ = 69.3, P < 0.0001) and was highest in backwaters (Figs. 3*b* and 3*c*) followed by impounded areas and lowest in the main and side channels. Seasonal effects were not but a significant season × aquatic area interaction effect ($F_{[6,335]}$ = 2.65, P < 0.016) reflected high rates in backwaters and impounded areas during summer and fall, a different pattern during spring and winter, and variation in the specific ranking of the backwaters and impounded areas by season. Spring flooding tended to equalize rates across all aquatic areas, particularly during 2001. Surprisingly, flooding during spring 2001 resulted in DEA rates only slightly higher (1.85 ± 0.54 μg N·cm^{-2}·h^{-1}) than that measured during the preceding winter (1.64 ± 0.26 μg N·cm^{-2}·h^{-1}). Average DEA was significantly lower in 2001 compared with 2000 (P < 0.0001) (2000: 11.8 ± 0.66 μg N·cm^{-2}·h^{-1}; 2001: 3.2 ± 0.30 μg N·cm^{-2}·h^{-1}).

Regression models predicting DEA consistently included

Fig. 3. Aquatic area and seasonal distribution (mean ± 1 SE) of (*a*) U-DEN during 2001, (*b*) DEA during 2000, and (*c*) DEA during 2001 in Navigation Pool 8. Asterisks indicate no data. Cross-hatched bars, backwaters; hatched bars, side channels; solid bars, main channel; open bars, impounded areas.



a combination of factors known to drive denitrification (N and sediment C) and microbial respiration (temperature) (Table 3). For example, in backwater areas, DEA was strongly affected by ambient NO_3^- concentration or factors controlling NO_3^- concentration (e.g., distance from main channel or rate of nitrification) and water temperature and sediment C (bulk density) in 2001 (2000: R^2 = 0.51; 2001: R^2 = 0.47). DEA in impounded areas was predicted by exchangeable NH_4 and sediment bulk density during 2000 (R^2 = 0.13) but by temperature, NO_3^- concentration, and distance from the main channel in 2001 (R^2 = 0.75). DEA in the main channel was related to NO_3^- concentration and temperature in 2000 (R^2 = 0.56), while in 2001, both surface water NH_4^+ and exchangeable NH_4^+ in sediments (R^2 = 0.57) were more important. Side-channel DEA rates were dependent (R^2 = 0.36) on distance from the main channel and sediment C (volatile mass) in 2000 and on sediment exchangeable NH_3 (R^2 = 0.57) in 2001. The model for the entire pool during 2000 included distance to channel, surface water NO_3^-, sediment exchangeable NH_4, and bulk density (R^2 = 0.35, P < 0.0001) and in 2001 included surface water NO_3^-, water temperature, nitrification rate, and bulk density (R^2 = 0.51, P < 0.0001). Variation in DEA was dependent on variation in nitrification most strongly during summer 2001 (R^2 =

Table 3. Regression models estimating DEA (μg N·cm^{-2}·h^{-1}) in four aquatic areas of Navigation Pool 8.

Aquatic area	Year	Regression model	*n*	R^2	Overall *P*
Backwater	2000	–0.0006(dist) – 9.7(bulkD) – 1.3(temp) + 53.6	76	0.51	<0.0001
	2001	0.016(nitrification) – 0.21(NO_3^-) – 1.61(bulkD) + 3.9*	49	0.47	<0.0001
Impounded	2000	0.36(x_amm) – 9.6(bulkD) + 26.1	65	0.13	0.002
	2001	0.18(temp) – 1.03(NO_3^-) + 0.004(dist) + 0.6	75	0.33	<0.0001
Main channel	2000	–0.98(NO_3^-) + 0.06(temp) + 0.88*	15	0.56	0.008
	2001	*3.68(s_NHx) + 0.11(x_amm) – 0.13*	28	0.57	<0.0001
Side channel	2000	–0.002(dist) + 21.7(v_mass) + 0.35*	23	0.36	0.011
	2001	0.19(x_amm) + 0.08*	26	0.57	<0.0001
Combined	2000	–18.0(bulkD) – 0.005(dist) – temp(0.36) + 46.9	179	0.35	<0.0001
	2001	–1.59(bulkD) – 0.21(NO_3^-) + 0.005(nitrification) + 0.02(temp) + 3.5*	178	0.51	<0.0001

Note: Models were developed with a stepwise selection technique, and parameter estimates are significant at the 0.05 level. dist, distance from channel (m); bulkD, sediment bulk density (g·cm^{-3}); temp, water temperature (°C); nitrification (μg N·cm^{-2}·h^{-1}); NO_3^-, surface water NO_3^- (mg·L^{-1}); x_amm, sediment exchangeable ammonium (mg·g^{-1}); s_NHx, surface water ammonium (mg·L^{-1}); v_mass, volatile mass of sediments (mg·g^{-1}).
*log(DEA + 1) transformed.

0.22, $P < 0.0001$) and at a low level ($R^2 = 0.07$, $P < 0.0001$) across all dates and habitats.

U-DEN averaged 0.19 ± 0.023 μg N·cm^{-2}·h^{-1}, ranging from 0.026 ± 0.013 μg N·cm^{-2}·h^{-1} in backwaters during fall to 0.40 ± 0.063 μg N·cm^{-2}·h^{-1} in backwaters during spring. U-DEN varied significantly among seasons ($F_{[2,175]} = 8.13$, $P = 0.0004$), but the response was dependent on aquatic area ($F_{[6,175]} = 2.67$, $P = 0.017$). For example, the highest rates were measured in backwaters, impounded areas, and side channels during the spring flood 2001, while rates tended to be highest in the main channel during summer (Fig. 3*a*). Rates were lowest in backwaters and impounded areas during fall. Regression analysis revealed that U-DEN rates were strongly dependent on NO_3^- concentrations in backwaters ($R^2 = 0.78$, $P < 0.0001$) and NH_4^+ in the main channel ($R^2 = 0.66$, $P < 0.0001$). No significant model could be developed for impounded areas and side channels. Pool-wide U-DEN rates were predicted with a combination of NO_3^- concentrations, distance from the channel, and sediment bulk density ($R^2 = 0.35$, $P < 0.0001$).

Limitation experiment

Denitrification was nearly undetectable in the unamended control of each sediment type (Fig. 4). NO_3^- additions resulted in a 30-fold increase in denitrification rates over controls in all sediments, regardless of sediment C levels (overall mean ± SE = 21.8 ± 1.13 versus 0.69 ± 0.22 μg N·cm^{-2}·h^{-1}). Glucose additions had little effect on denitrification accept in low-C sediments. The C + NO_3^- treatment showed a slight but significant reduction in denitrification in all sediments (20.4 ± 0.34 μg N·cm^{-2}·h^{-1}). Intermediate C sediments showed the strongest response to NO_3^- and C additions and high-C sediments generally showed the weakest response.

NO_3^- budget

NO_3^- loss from the reach through denitrification was highest during summer (26.6 t·day^{-1}), with NO_3^- losses during the other seasons of the year amounting to another 51 t·day^{-1} (Fig. 5). Greater than 80% of the NO_3^- losses occurred in impounded and backwater areas. We estimate that 7% (6939 t·year^{-1}) of the total annual NO_3^- input to Pool 8 (99 922 t·year^{-1}) was removed through denitrification (Fig. 6). NO_3^- removal through denitrification was nearly equal to that produced through nitrification (6986 t·year^{-1}); unquantified processes removed 13% of the total NO_3^- load. Mass balance shows that Pool 8 functioned as a sink for

Fig. 4. Denitrification (mean ± 1 SE) resulting from addition of C, NO_3^-, or C + NO_3^- to sediment of high, medium, or low C content ($n = 4$). Letters above the bars indicate significant differences ($P < 0.05$) among treatment means; different letters indicate significantly different means.

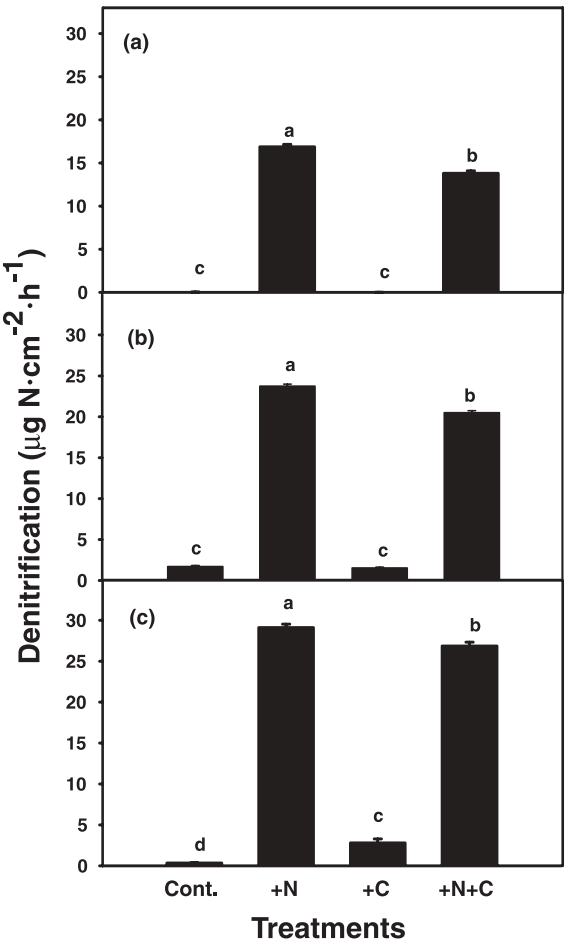
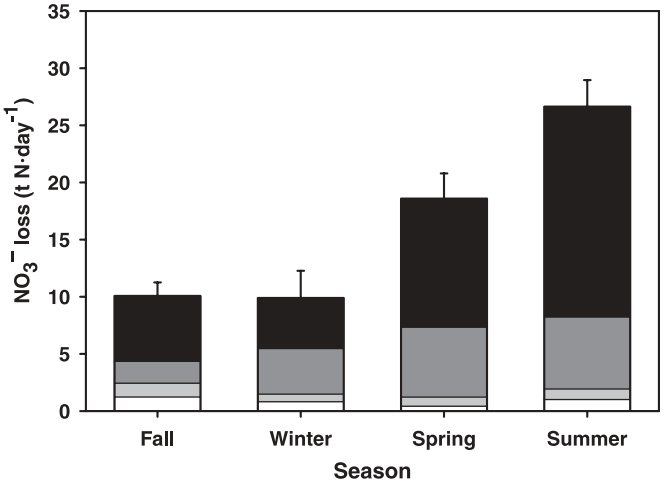


Fig. 5. Seasonal NO_3^- loss (mean ± 1 SE) for aquatic areas in Navigation Pool 8. Darker shaded bars, backwaters; lighter shaded bars, side channels; open bars, main channel; solid bars, impounded areas.



NO_3^-, removing about 20% of the total input throughout the year, but denitrification accounted for little of the loss.

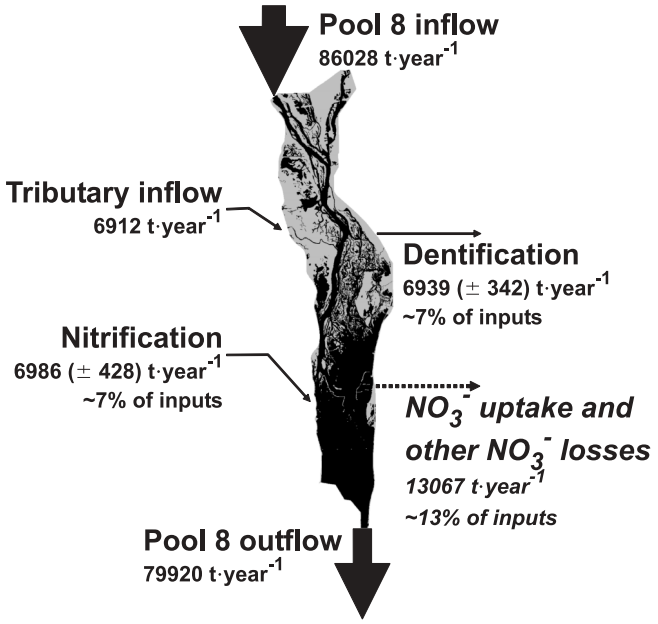
Discussion

Rates of denitrification in the UMR were similar to or higher than (mean EDR = 105 μg $N \cdot cm^{-2} \cdot h^{-1}$) rates in other sediment systems. Mean rates ranged from 0.14 μg $N \cdot cm^{-2} \cdot h^{-1}$ (spring 2001 in the main channel) to 1.97 μg $N \cdot cm^{-2} \cdot h^{-1}$ (summer 2001 in backwaters). Sediment from other river and lake systems also exhibits a wide range of denitrification rates depending on nutrient enrichment, water temperature, and C availability. For example, Seitzinger (1988) reported denitrification rates up to 0.48 μg $N \cdot cm^{-2} \cdot h^{-1}$ in a survey of river data, while oligotrophic–mesotrophic lakes ranged from 0.007 to 0.08 μg $N \cdot cm^{-2} \cdot h^{-1}$ and eutrophic lakes ranged from 0.06 to 0.24 μg $N \cdot cm^{-2} \cdot h^{-1}$. High denitrification rates (0.18–10.2 μg $N \cdot cm^{-2} \cdot h^{-1}$) estimated in sediments of the South Platte River, Colorado, were likely the result of consistently high NO_3^- concentrations in the water column (average >5 mg $N \cdot L^{-1}$), minimal spatial heterogeneity in the channel form, or hydraulic isolation (relative to the UMR), resulting in high NO_3^- delivery throughout much of the channel (Sjodin et al. 1997). Pattinson et al. (1998) reported rates in the Wiske–Swale–Ouse River system in the United Kingdom ranging from 2.5 μg $N \cdot cm^{-2} \cdot h^{-1}$ in upstream reaches to over 91.7 μg $N \cdot cm^{-2} \cdot h^{-1}$ in downstream reaches. The Swale–Ouse River system also exhibited a trend toward high rates in spring owing to both high NO_3^- and increasing water temperatures. This pattern is in contrast with that found in the UMR, where cold winter water temperatures likely keep bacterial metabolism and denitrification rates low until the river warms after spring floods.

Seasonality and river discharge

The dynamics of denitrification in the UMR are controlled by interacting factors of NO_3^- delivery (through variation in river stage), sediment characteristics, and water temperature. Variation in river discharge, particularly flooding and low flows, is critical for redistribution and depletion of NO_3^-.

Fig. 6. NO_3^- budget for Navigation Pool 8 (modified from Strauss et al. 2004).



Variation in river stage is a relatively predictable consequence of climate and season, and as such corresponds to fairly predictable variation in water temperature. Seasonal patterns of denitrification reflect these interactions. Winter (2001) DEA was low and likely resulted from cold water temperatures, not a lack of NO_3^-, and rates during spring were variable, depending on the extent of flooding. In spring 2000, no flood occurred and rates were lower than those in the subsequent summer (no data exist for a winter 2000 comparison). In spring 2001, with record flooding, rates were extremely low (similar to those of the preceding winter), but NO_3^- concentrations were nearly double that in winter and temperatures about 18 °C higher.

The record flood of spring 2001 appeared to have a lasting effect on the Navigation Pool 8 because DEA in subsequent months was significantly reduced relative to the previous year. Because water temperatures and dissolved oxygen concentrations were not significantly different between the two years (both slightly elevated during 2001), physical disturbance and erosion of the sediment surface likely resulted in reduced populations of denitrifying bacteria.

Summer or fall DEA tended to be higher than spring or winter DEA (depending on year), being strongly dependent on antecedent river stage. This river stage phenomenon is reflected in the regression models for DEA where discharge in the previous 21 days was a common predictor. During 2000, fall sampling occurred several weeks after flooding and sampling in summer 2001 occurred on the descending leg of a record flood. Higher summer temperatures result in elevated microbial metabolism such that NO_3^- is metabolized more rapidly than in cooler seasons, particularly in areas of the floodplain with abundant C (backwaters and impounded areas) (Seitzinger 1988; Pfenning and McMahon 1996; Pattinson et al. 1998).

Backwater lakes of this reach of the UMR often exhibited high DEA rates. High DEA measurements in backwater and impounded habitats during spring, summer, and fall 2000 in-

dicate that these relatively high C environments contained sufficient NO_3^- within the previous 3–4 weeks (Groffman et al. 1999) and that antecedent water temperatures were sufficient to promote bacterial activity and population growth. In the month previous to all three sampling periods, river discharge was elevated and likely resulted in increased NO_3^- concentrations across the pool. Discharge during spring 2000, while high, was not near historical levels because of an unseasonably warm, dry winter and sparse snow pack. Water temperatures during the preceding winter were also slightly elevated compared with normal. These factors likely combined to result in larger than normal bacterial populations and DEAs that were much greater during spring 2000 than during spring 2001. While NO_3^- concentrations were relatively high across the entire pool during spring 2001, we suspect that denitrifier populations were still reduced from the cold winter of 2000–2001 and too low to metabolize substantial quantities of NO_3^- (Pelletier et al. 1999).

Geomorphology and flow

Patterns of river discharge and floodplain geomorphology are critical in determining sediment C characteristics through processes of erosion and deposition, which in turn determine the environmental setting for sediment-based N cycling. Low-energy depositional areas tend to accumulate organic matter, support growth of aquatic macrophytes, and provision microbial metabolism with ample C. The patterns of denitrification exhibited by particular aquatic areas (river habitats) were consistent through seasons and reflect these linkages among biogeomorphic processes. For example, main channels are high-energy erosional environments where sediments contain little organic C, scour prevents rooting of most plants, but surface water NO_3^- concentrations are high. Here, low infiltration of surface water (i.e., NO_3^-) into sediments, low sediment C, and mostly aerobic conditions keep denitrification rates low.

In contrast, backwater lakes are low-energy environments, with little erosion except during floods, containing high-C sediments and high plant densities. DEA is extremely high in these sediments, suggesting that a NO_3^- source exists (probably nitrification) and that much higher rates of ambient denitrification are possible with increased delivery of NO_3^- (e.g., summer floods).

Impounded areas are hybrids between channels and backwaters and conditions are sufficient to support high denitrification rates relative to channels and, occasionally, backwaters. Sediment C content in impounded areas tends to be slightly greater than in channels and NO_3^- availability is greater than in backwaters, resulting in conditions adequate for denitrification.

Microbial cycling of N in the UMR is, then, clearly linked to pool-wide spatial patterns of NO_3^- and C distributions. Geomorphology and river discharge interact to regulate concentrations of water column NO_3^- and sediment C. Hydraulic isolation of C-rich areas during periods of low river discharge limits the supply of NO_3^- and results in NO_3^- limitation of denitrification. Conversely, high river discharge (increased hydraulic connectivity) distributes NO_3^--rich water to C-rich backwaters and promotes denitrification (particularly during warm seasons).

Similar patterns of NO_3^- dynamics were found in other floodplain river systems. For example, Missouri River floodplain lakes lacking a direct connection to flowing channels exhibit strikingly low concentrations of N in relation to the main channel (Knowlton and Jones 1997). Lakes with greater connectivity typically contain higher concentrations of N. Oronoco River floodplain lakes exhibit a similar pattern of connectivity driving N dynamics (Lewis et al. 2000). Deposition of N-rich particles on floodplains during floods is well documented and likely plays an important role in resupplying N-depleted sediments (e.g., Brunet et al. 1993). Flood duration is also an important determinant of floodplain denitrification by controlling the timing and duration of soil saturation (Pinay et al. 2000).

The interplay between NO_3^- supply and sediment organic matter has been noted in other river systems. For example, C-rich riverine wetlands of northern Minnesota and Wisconsin were found to be NO_3^- limited for denitrification during summers while exhibiting high potential denitrification (Johnston et al. 2001). In addition, sediments nearer to rivers tended to contain higher NO_3^- and increased rates of denitrification. Denitrification rates in the Wiske–Swale–Ouse River system in the United Kingdom were dependent on both water column NO_3^- and sediment percent moisture (a covariate of sediment C; Garcia-Ruiz et al. 1998*a*, 1998*b*).

Coupled nitrification and denitrification

Relatively high DEA (and low U-DEN) in backwater areas suggests that a NO_3^- source supports relatively high rates of enzyme production, even when surface water concentrations are near detection limits. Groundwater is a potential source of NO_3^- in many areas of Pool 8, but the distribution of such inputs is limited to a few isolated areas (R. Hunt, US Geological Survey, 8505 Research Way, Middleton, WI 53562, USA, personal communication). Nitrification is a more likely source of NO_3^- driving DEA. Using data from Strauss et al. (2004), we show that nearly 15% (R^2 range 0 – 0.87) of the variation in DEA is related to variation in rates of nitrification across the pool. Even though nitrification is often limited by oxygen in backwater sediments in the UMR (Strauss et al. 2004), nitrification clearly plays a role in supporting denitrification by provisioning sediments with sufficient NO_3^- to stimulate enzyme production. Coupling of these processes is relatively common (Jenkins and Kemp 1984; Seitzinger 1988) and may be important in the UMR to support denitrification, particularly in aquatic areas isolated from the main channel.

Management implications

In absolute terms, large quantities of NO_3^- are being removed from the UMR in Pool 8 by denitrification and other processes, but it is clear that the NO_3^- processing capacity of the river is being overwhelmed by the staggering NO_3^- load from upstream sources (Battaglin et al. 2001). In the upper third of the basin, much of the floodplain remains physically connected to the channels, but insufficient lateral movement of main channel water limits NO_3^- delivery and NO_3^- uptake in C-rich backwater areas.

If a management goal is to reduce NO_3^- flux from the UMR basin (Mitsch et al. 2001), then rerouting some main

channel water through backwater areas in the UMR would aid in achieving this goal. We suspect, however, that the NO_3^- load carried by the Mississippi River is so great that there is insufficient sediment surface area to facilitate removal of more than a small fraction of the current N load (Alexander et al. 2000). Given that only 30–40% of the total NO_3^- load reaching the Gulf of Mexico originates in the UMR basin, optimal denitrification in the UMR would only reduce that load by about 5–10%. Furthermore, rerouting of water into backwater lakes must be done cautiously because of the potential for unintended results (e.g., cyanobacteria blooms, anoxia, and fish kills; Poirrer and King 1998). Another river management strategy, water level manipulations resulting in large-scale sediment drying/rewetting, holds some promise for enhancing N removal capacities of river sediments. Preliminary results from such manipulations in Pool 8 show reductions in N content of desiccated and rehydrated sediments (W.B. Richardson et al., unpublished data). Clearly, a constellation of management strategies, both in upland landscapes and in rivers, must be undertaken to significantly reduce downstream flux of N from the UMR system.

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